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Non-invasive Measurement of Blood Pressures in the Yucatan Micropig (*Sus scrofa domestica*), With and Without Midazolam-Induced Sedation

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Current literature suggests that the effects of midazolam, a water-soluble benzodiazepine, on blood pressure in swine are minimal. The hypothesis of the study reported here was that a light sedative dose would induce a decrease in blood pressure in this species. Healthy female Yucatan Micropigs (n = 20), 16 to 30 (mean, 22) kg, aged four six months, were individually placed in a humane restraint sling and allowed to acclimate. Systolic (SBP), diastolic (DBP), and mean (MBP) blood pressures (mmHg) and heart rate (HR; beats per min [bpm]) were measured by use of oscillometry. The pressure cuff was placed at the base of the tail, and five sets of values were recorded at five-min intervals, beginning at 10 and ending 30 min after cuff placement. Following a three- to four-day rest period, this procedure was repeated with the addition of a dose of 0.5 mg of midazolam HCl/kg of body weight given intramuscularly at the time of cuff placement. A paired one-way Student's *t*-test was used to compare the means of the five measures between control and midazolam treatment. Mean (± SD) differences for SBP, DBP, MBP, and HR were 18.9 (± 3.97), 17.8 (± 5.27), and 18.6 (± 5.09) mmHg and 20.7 (± 3.73) bpm, respectively. All four parameters were significantly reduced in the midazolam-sedated group ($P < 0.001$). The maximal decrease in SBP, DBP, and MBP occurred at 15 and 20 min after dosing. Mean values based on the means of the five measures were 128 (± 12.6), 80 (± 9.4), and 99 (± 9.2) mmHg and 135 (± 17.4) bpm, and 109 (± 15.4), 63 (± 12.6), and 80 (±13.6) mmHg and 115 (± 15.5) bpm for SBP, DBP, MBP, and HR in the control (n = 20) and midazolam (n = 20) groups, respectively. The control values can serve as normal oscillometric values for this age, sex, and breed of Micropig. We conclude that midazolam, given intramuscularly at a sedative dosage, negatively affects cardiovascular parameters measured by use of a blood pressure cuff, in sexually mature female Micropigs, compared with values in untreated pigs, which is similar to reports for humans.

During the past five years we have been evaluating and developing the Yucatan Micropig (*Sus scrofa domestica*) as a model of menopause with respect to the cardiovascular system and atherogenesis. Minipigs and farm pigs have been used as models of atherosclerosis for several decades; however, there are minimal data on how this animal model responds to estrogen deficiency and replacement when fed a high fat, high cholesterol diet. Recently at the 11th annual meeting of the North American Menopause Society (NAMS), we presented data documenting that the ovariectomized Micropig receiving conjugated equine estrogen replacement therapy (ERT) had 51% reduction in coronary artery atherosclerosis, compared with that of estrogen-deficient (ovariectomized) controls (1). This is consistent with the response in several other animal models including chickens, white Carneau pigeons, rabbits, and particularly cynomolgus macaques (2). This also supports use of this model to study alternatives to ERT and hormone replacement therapy (HRT), including synthetic conjugated estrogens, phytoestrogens, selective estrogen receptor modulators (SERMS), or tissue selective estrogens (TSEs).

Blood pressure is an important risk factor for development and progression of coronary atherosclerosis and other cardiovascular diseases (3). Therefore it is beneficial for use and development of this model to have normal values and established techniques to collect and evaluate values for this parameter in this species. Use of non-invasive techniques is desirable for this chronic model simply to avoid the typical complications associated with catheterization. Use of a short-acting sedative (midazolam) facilitates acquisition of blood pressure values in pigs since movement by the animal prevents obtaining a reading. Midazolam, one of many benzodiazepines, was chosen because it reportedly has minimal effects on cardiovascular function in swine (4, 5). However, on the basis of effects of other drugs in this class and the information for humans in the product (VERSED, Roche Pharmaceuticals, Nutley, N.J.) insert (6), we hypothesized that it would lower blood pressures in these animals.

Materials and Methods

Animals and study conditions: The animals studied were 20 healthy sexually intact female Yucatan Micropigs, 16 to 30 (mean, 22) kg in body weight, aged four to six months at the time. They were obtained from Charles River Laboratories, Wilmington, Mass., and were allowed to condition for three weeks in their new environment at 22°C ± 1°C and 30 to 70% relative humidity. The lighting was 12-h light and dark cycles, with approximately 15 air changes/h. Pigs were housed individually in 30 ft^2 pens on raised fiberglass slatted floors; they were fed Teklad Mini-swine breeder sterilizable diet (No. 7037, Harlan Teklad, Madison, Wis.) and had water available from an automatic watering system. The program and facilities were approved by AAALAC International, and the institutional animal care and use committee approved the methods used in this laboratory investigation.

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Parameter determinations: The equipment consisted of a SpaceLabs Medical PC Scout Monitor 90309 with the adult/neonatal NIBP module and software (SpaceLabs Medical, Inc., Redmond, Wash.). The neonatal configuration with a No. 2 neonatal pressure cuff placed at the base of the tail was used while each pig was resting comfortably in an appropriately sized swine hammock-sling. The monitor was set up to measure systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP), and heart rate (HR).

In turn, each pig was placed in the swine sling and carefully moved to the same empty, quiet, dimly lit room, with consistent temperature and positioned near the blood pressure monitor. The pig was examined to ensure that it was comfortable in the sling, then the cuff was placed at the base of the tail (coccygeal artery). Proper function of the monitoring system was validated, and the equipment was programmed to read at 5-min intervals, with the first at 10 min after cuff placement. The technician then departed the room and observed the pig and monitor through the window in the door. The 10 min from cuff placement until the first reading was the acclimation period. Once the five readings had been taken, the technician entered the room, removed the cuff, and returned the pig to its original pen. The strategy was to have the pig in the sling for as short a period as possible, thus keeping the handling that each pig experienced consistent throughout the study. Following a three- to four-day rest period, this procedure was repeated for the same 20 pigs in the same room, using the same equipment and technician, with the addition of a dose of 0.5 mg of midazolam/kg of body weight given intramuscularly at the time of cuff placement.

Statistical analysis: A paired one-way Student's *t*-test was used to compare the means of the five measures between control and midazolam treatment. A one-tailed test was chosen because a difference in one direction, a decrease, in all four parameters was hypothesized to result from midazolam treatment. Additionally, the mean values, with and without midazolam treatment, at each time point (10, 15, 20, 25, and 30 min) were compared with the same test. In all instances, n = 20 and a P value < 0.05 was considered significant.

Results

Midazolam treatment of the 20 Micropigs produced values for all four parameters (SBP, DBP, MBP, HR) that were significantly lower than those obtained when the same animals were not treated (normal controls) ($P < 0.001$, Table 1). Mean with standard error over time values are illustrated (Figs. 1-4). Specifically, the means of the five measures ($P < 0.001$) and at each time point checked ($P < 0.05$), with the one exception being the SBP at 10 min, of each of the four parameters were significantly lower in association with midazolam treatment. The reduction in blood pressures was maximal at the 15- and 20-min time points, and a return toward control values was evident by 30 min after dosing. The HR continued to decrease throughout the 30-min study. Positioning the pressure cuff at the base of the tail worked well in this study because the pigs were less likely to move their tail than limbs while restrained in the hammock-sling.

Table 1. Mean (SD) values for the five measures of blood pressure and heart rate in pigs, with (n = 20) and without (n = 20) midazolam treatment of the same 20 Micropigs

	SBP (mmHg)	DBP (mmHg)	MBP (mmHg)	HR (bpm)
Normal (n = 20)	128 (12.60)	80 (9.40)	99 (9.20)	135 (17.40)
+ Midazolam (n = 20)	109 (15.40)	63 (12.60)	80 (13.60)	115 (15.50)
Mean difference	18.9 (3.97)	17.8 (5.27)	18.6 (5.09)	20.7 (3.73)
P value	<0.001	<0.001	<0.001	<0.001

SBP = systolic blood pressure; DBP = diastolic blood pressure; MBP = mean blood pressure; HR = heart rate; and bpm = beats per minute.
$P < 0.05$ was considered significant.

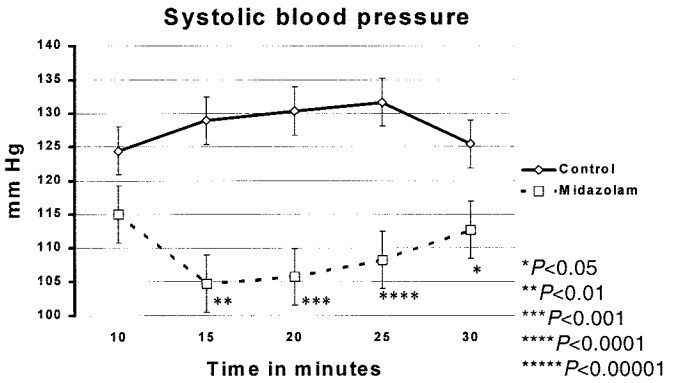

Figure 1. Systolic blood pressure plotted over time in pigs, with (n = 20) and without (n = 20) midazolam treatment (0.5 mg/kg of body weight, i.m.) of the same 20 Micropigs. Data shown as mean ± SEM by use of paired one-way Student's *t*-test ($P < 0.05$ was considered significant).

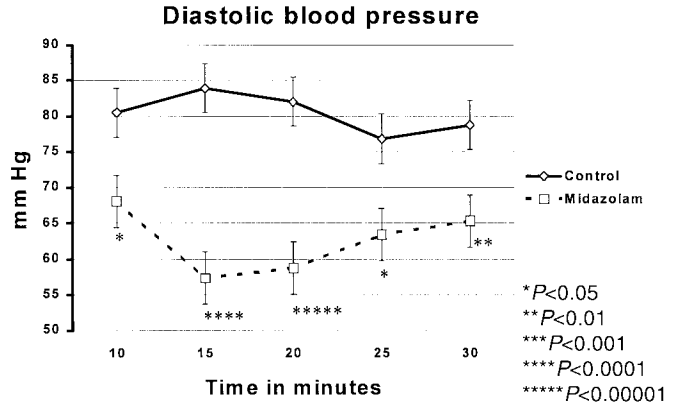

Figure 2. Diastolic blood pressure plotted over time in pigs, with (n = 20) and without (n = 20) midazolam treatment (0.5 mg/kg, i.m.) of the same 20 Micropigs. Data shown as mean ± SEM by use of paired one-way Student's *t*-test ($P < 0.05$ was considered significant).

Discussion

Normal blood pressures in the context of this study were qualified as blood pressures measured on swine resting/restrained comfortably in a sling. Values collected from a pig in a hammock-sling that does not fit properly or a pig that was resting freely in the pen would be expected to be different. Therefore factors, such as type and duration of restraint, must be kept as stress free and as short and certainly consistent or controlled as possible in this type of study. The presence of a technician sitting next to the pig during a reading and noises coming from the next room are potentially additional sources of variation. In this study, a conscious effort was made to eliminate these obvious sources of variation.

Benzodiazepines are a class of drug that act on benzodiazepine receptors (7), which in turn decrease anxiety and are expected to induce some degree of cardiorespiratory depression

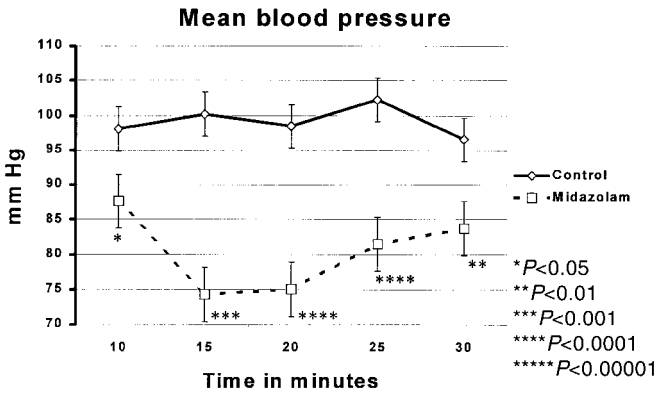

Figure 3. Mean blood pressure plotted over time in pigs, with (n = 20) and without (n = 20) midazolam treatment (0.5 mg/kg, i.m.) of the same 20 Micropigs. Data shown as mean ± SEM by use of paired one-way Student's *t*-test ($P < 0.05$ was considered significant).

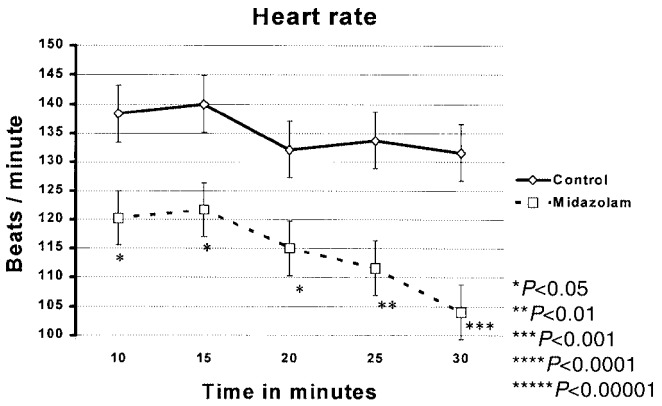

Figure 4. Heart rate plotted over time in pigs, with (n = 20) and without (n = 20) midazolam treatment (0.5 mg/kg, i.m.) of the same 20 Micropigs. Data shown as mean ± SEM by use of paired one-way Student's *t*-test ($P < 0.05$ was considered significant).

(8). The dose of midazolam used in this study was chosen because it induces adequate sedation for minor procedures, such as echocardiography and blood pressure measurements. Additionally, it has prior references in the literature. Systolic blood pressure measured by use of Doppler imaging in 6 mixed-breed pigs (4 males, 2 females, 18 to 29 kg) decreased by 11 mmHg from control values by 30 min after dosing (0.5 mg of midazolam/kg, i.m.) (4). Heart rate also decreased in the pigs of this study by 22 bpm by the same time point; however, neither value was statistically significant. This could be due to inadequate statistical power (n = 6) (type-II error) and/or that maximal reduction was at 20 min. Measurements were taken at 3, 5, 10, 15, 30, and 60 min after dosing. These results indicated maximal sedation at 15 min and minimal alterations in cardiovascular function. A previous report (5) indicated that mean aortic pressures measured via aortic vascular catheters and pressure transducers in conventional male Yorkshire swine (23 to 30 kg) (n = 5) significantly increased after sequentially (0.1 mg/kg increments at 1-h intervals) increasing intravenously administered doses reached 0.5 mg/kg. Heart rate was significantly decreased at the same point. The values were collected at 15 min after dosing. The discrepancy in these findings may possibly be due to the cumulative effects of repeated intravenously administered doses, and that the pigs had been in their slings in excess of four h when the increase in blood pressure became significant. The technique differences (surgically implanted vascular catheters versus oscillometry or Doppler) may also have been a factor. Additionally, sex, breed, and age differences in cardiovascular responses to midazolam sedation cannot be ruled out. The data presented here that indicate significantly lower blood pressures and HR associated with midazolam treatment in a group of 20 females have the advantage of increased statistical power above that provided in the two prior swine studies discussed. These results are also entirely consistent with effects in humans described in the product insert (6) and published by various authors. (8, 9)

In conclusion, normal blood pressure and HR values for this breed, sex, age, and technique (oscillometry) are given and can be used as reference values. Also, positioning the pressure cuff at the base of the tail is a valid technique for collecting blood pressure data from swine in slings. Finally, the data presented here indicate that the values for SBP, DBP, MBP, and HR associated with midazolam sedation at a dosage of 0.5 mg/kg given intramuscularly will be significantly lower than normal and this should be realized when collecting and evaluating cardiovascular physiologic measurements.

Acknowledgments

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References

1. **Goodrich, J. A., T. B. Clarkson, M. M. Swindle, A. Jenkins, M. J. Del Signore, and T. J. Lyons.** 2000. Levormeloxifene effects on coronary artery atherosclerosis and the uterus of Yucatan Micropigs. Menopause **7:**430.
2. **Adams, M. R., S. A. Washburn, J. D. Wagner, J. K. Williams, and T. B. Clarkson.** 1999. Arterial changes: estrogen deficiency and effects of hormone replacement, p. 377-384. *In* R. A. Lobo (ed.), Treatment of the postmenopausal woman: basic and clinical aspects, 2nd ed. Lippincott Williams & Wilkins, Philadelphia.
3. **MacMahon, S.** 2000. Blood pressure and the risk of cardiovascular disease. N. Engl. J. Med. **342:**50-51.
4. **Bustamante, R., and A. Valverde.** 1997. Determination of a sedative dose and influence of droperidol and midazolam on cardiovascular function in pigs. Can. J. Vet. Res. **61:**246-250.
5. **Smith, A. C., J. L. Zellner, F. G. Spinale, and M. M. Swindle.** 1991. Sedative and cardiovascular effects of midazolam in swine. Lab. Anim. Sci. **41:**157-161.
6. **Roche Laboratories.** 1999. Versed (midazolam HCL) product insert. Roche Pharmaceuticals, Nutley, N.J.
7. **Tallman, J. F., S. M. Paul, P. Skolnick, and D. W. Gallager.** 1980. Receptors for the age of anxiety: pharmacology of the benzodiazepines, Science **207:**274-281.
8. **Sunzel, M., L. Paalzow, L. Berggren, and I. Erriksson.** 1988. Respiratory and cardiovascular effects in relation to plasma levels of midazolam and diazepam. Br. J. Clin. Pharmacol. **25:**561-569.
9. **Langlois, S., J. H. Kreeft, G. Chouinard, A. Ross-Chouinard, S. East, and R. I. Ogilvie.** 1987. Midazolam: kinetics and effects on memory, sensorium, and haemodynamics. Br. J. Clin. Pharmacol. **23:**273-278.